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CULTURES OF TUBERCLE BACILLI ISOLATED FROM MILK.*¹

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IN an examination of the milk supply of New York City made by me over a year ago, 17 samples among 107 of milk retailed from 40-quart cans were found to contain tubercle bacilli. A full report of this study, together with a consideration of the welfare of the children who drank this contaminated milk, has been published elsewhere.² In the course of this work it was considered that it might be of interest to isolate some of these strains of tubercle bacilli, and to determine their cultural and pathogenic characteristics. It was to be expected, considering the source of the material, that probably all the strains would prove to be bovine in type. However, there seemed a possibility, owing to the great degree of exposure to which such milk is subject in its journey from the cow to the retailer, that contamination with bacilli from human source might occur. In the hope of encountering such a case cultures were undertaken. It seemed also of interest to inquire whether the cultures would conform strictly to definite types or whether intermediate varieties, such as have been met with by some others, would be isolated.

The technique employed was, in brief, as follows:

Ten c.c. of milk was centrifugalized, and 1 c.c. of the cream injected into a guinea-pig; 1 c.c. of the lowest skimmed milk was inoculated into another guinea-pig. In some cases one of these pigs developed tuberculosis, in other instances both of them. Of the cultures which I now report, five were made from pigs inoculated with cream, three from animals inoculated with sediment. I shall not detail the method of obtaining cultures, as it is the one generally in use, and similar to that employed by me in previous studies.³

In some cases coagulated dog serum was used as a medium, as first suggested by Theobald Smith, but in most instances the tissue was transferred directly to Dorset's egg media. In two instances, not included in this series, culture was attempted but failed; in two others the growth was very scanty, and after the third generation the strain was lost.

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¹ Read before the New York Pathological Society, January 13, 1909.

² *Jour. Amer. Med. Asso.*, 1909, 51, p. 1011.

³ *Archiv. Pediat.*, 1908, p. 31; *Amer. Jour. Med. Sci.*, 1908, 136, p. 1831.

The results are given in the appended table. A review of this summary shows that seven cultures induced a fatal generalized tuberculosis in the rabbit, whereas one possessed but slight virulence for this species. For these tests intravenous inoculations of standard emulsions were employed, excepting in the last case, where it will be noted that 1 mg. of culture was inoculated. This strain was isolated and studied by Dr. Woglam and Dr. Krumweide, whom I take pleasure in thanking for this work.

The cultures I-VII must be classed as bovine in type, not only on account of the marked virulence to rabbits which they exhibited, but also because of their cultural characteristics. They grew very sparsely, showing for some generations only a fine veil-like growth, which it was very difficult or impossible to transfer successfully to glycerin broth. They were markedly similar in all these properties, only one standing apart from the others in that it showed a more abundant growth in the first generation. However, even this strain did not grow profusely when compared with cultures of the human variety.

Culture VIII is of especial interest and seems worthy of detailed description. The original material was obtained from a can of milk in a small grocery store. It was inoculated subcutaneously into two guinea-pigs, both of which developed generalized tuberculosis. From one of these animals tissue was inoculated into another pig (No. 855), from which cultures were made upon egg media about two months later. In order not to lose the material, tissue was once more transferred into a pig (No. 972), and further cultures carried out after the same interval. These two sets of cultures proved to be identical; after three weeks a vigorous confluent growth was obtained on glycerin egg. These results were so unexpected that a rabbit (No. 625) was inoculated with one of these strains, and cultures were made from its tissues. By using the rabbit instead of the highly susceptible guinea-pig we made certain of selecting for culture the most virulent bacilli. These strains also grew vigorously in a manner characteristic of bacilli of the human type. Reference to the virulence tests of the table shows that this strain (Culture VIII) in strong contrast to all the others, possessed but feeble virulence for rabbits. One of the rabbits when inoculated weighed 1,740 gm.,

and when killed had gained 550 gm., the other weighed 1,710 gm., and now, after a period of 63 days, weighs 1,820 gm. Thus this culture, in contradistinction to the others, must be regarded as belonging to the human type, both on account of its facility for cultivation and its comparatively feeble virulence.

From a study of these eight cultures we must emphasize the sharp contrast which usually exists between tubercle bacilli of the human and those of the bovine type, isolated from cattle, a distinction which in the instances here reported was absolutely diagnostic and incapable of misinterpretation. Furthermore the results are instructive from another point of view, for, for the first time, they bring forth an instance where tubercle bacilli of the human variety have been isolated from milk and thus point out another source of danger from contamination by the tuberculous individual.

TABLE I.
DATA OF INTRAVENOUS INOCULATION OF RABBITS WITH PURE CULTURES OF TUBERCLE BACILLI.

	Total Age of Culture	Genera- tion	Age of Culture	Amount Inocu- lated	No. Rabbit	Result	General Remarks
Culture I ..	82 days	3d	22 days	0.5 c.c.	320	Chloroformed 21 days (ill)	General tuberculosis
" II ..	95 "	4th	20 "	0.5 c.c.	321	Died 18 days	" "
" III ..	76 "	3d	25 "	0.5 c.c.	325	" 16 "	" "
" IV ..	67 "	3d	28 "	0.4 c.c.	326	" 20 "	" "
" V ..	98 "	4th	21 "	0.5 c.c.	327	" 23 "	" "
" VI ..	79 "	3d	30 "	0.5 c.c.	331	Chloroformed 17 days (ill)	" "
" VII ..	69 "	3d	26 "	0.5 c.c.	332	Died 15 days	" "
" VIII {	21 "	3d	63 "	1 mg.	181	Killed 80 days	Few tubercles in lungs and kidneys
	21 "	2d	42 "	1 mg.	521	Alive 63 days	Gained in weight